FUSE

Fungal Systematics and Evolution

VOLUME 6
DECEMBER 2020
PAGES 39–53

doi.org/10.3114/fuse.2020.06.03

Two new species of the *Peronospora belbahrii* species complex, *Pe. choii sp. nov*. and *Pe. salviae-pratensis sp. nov*., and a new host for *Pe. salviae-officinalis*

M. Hoffmeister[1], S. Ashrafi[1], M. Thines[2,3,4]*, W. Maier[1]*

[1]Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Messeweg 11-12, 38104 Braunschweig, Germany
[2]Goethe University, Faculty of Biological Sciences, Institute of Ecology, Evolution and Diversity, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany
[3]Senckenberg Biodiversity and Climate Research Centre, Senckenberganlage 25, 60325 Frankfurt am Main, Germany
[4]LOEWE-Centre for Translational Biodiversity Genomics, Georg-Voigt-Str. 14-16, 60325 Frankfurt am Main, Germany

*Joint senior and corresponding authors, listed in alphabetical order of the first names: m.thines@thines-lab.eu; wolfgang.maier@julius-kuehn.de

Key words:
coleus
downy mildew
multi-gene phylogeny
new host report
new taxa
Peronosporaceae

Corresponding editor:
P.W. Crous

Abstract: The downy mildew species parasitic to *Mentheae* are of particular interest, as this tribe of *Lamiaceae* contains a variety of important medicinal plants and culinary herbs. Over the past two decades, two pathogens, *Peronospora belbahrii* and *Pe. salviae-officinalis* have spread globally, impacting basil and common sage production, respectively. In the original circumscription of *Pe. belbahrii*, the downy mildew of coleus (*Plectranthus scutellarioides*) was ascribed to this species in the broader sense, but subtle differences in morphological and molecular phylogenetic analyses using two genes suggested that this pathogen would potentially need to be assigned to a species of its own. In the present study, *Peronospora* species causing downy mildew on members of the *Mentheae*, including clary sage (*Salvia sclarea*), meadow sage (*S. pratensis*), basil (*Ocimum basilicum*), ground ivy (*Glechoma hederacea*) and coleus (*Plectranthus scutellarioides*) were studied using light microscopy and molecular phylogenetic analyses based on six loci (ITS rDNA, *cox*1, *cox*2, *ef1a*, *hsp*90 and *β-tubulin*) to clarify the species boundaries in the *Pe. belbahrii* species complex. The downy mildew on *Salvia pratensis* is shown to be distinct from *Pe. salviae-officinalis* and closely related to *Pe. glechomae*, and is herein described as a new species, *Pe. salviae-pratensis*. The downy mildew on *S. sclarea* was found to be caused by *Pe. salviae-officinalis*. This is of phytopathological importance, because meadow sage thus does not play a role as inoculum source for common sage in the natural habitat of the former in Europe and Asia, while clary sage probably does. The multi-gene phylogeny revealed that the causal agent of downy mildew on coleus is distinct from *Pe. belbahrii* on basil, and is herein described as a new taxon, *Pe. choii*.

Effectively published online: 3 March 2020.

INTRODUCTION

Over the past two decades several downy mildew diseases in medicinal plants and culinary herbs have been newly reported and led to economic losses. Prominent examples are *Peronospora somniferi* on opium poppy (Voglmayr *et al.* 2014), *Pe. belbahrii* on basil (Thines *et al.* 2009) and *Pe. salviae-officinalis* on common sage (Choi *et al.* 2009). The latter two species are closely related and belong to a clade we refer to as the *Pe. belbahrii* species complex. Apart from the two mentioned species, it is known to contain *Pe. elsholtziae* and *Pe. salviae-plebeiae* (Choi *et al.* 2009). Of the species in the complex, especially *Pe. belbahrii* and *Pe. salviae-officinalis* have proven to be destructive pathogens in the production of the respective crops. When downy mildew disease was first discovered on basil and common sage, it was mostly considered to belong to *Pe. lamii* (McMillan 1993, Gamliel & Yarden 1998, Plenk 2002, Hill *et al.* 2004, Belbahri *et al.* 2005, Liberato *et al.* 2006, Humphreys-Jones *et al.* 2008, Choi *et al.* 2009), according to the broad species concept advocated by Yerkes & Shaw (1959) for some downy mildew groups. However, this species concept is generally not appropriate for downy mildews as demonstrated by several phylogenetic studies over the past 20 years or so (for a review see Thines & Choi 2016). Specifically, it had been shown that the taxon *Pe. lamii* should be restricted to the downy mildew parasitizing *Lamium* spp. or *L. purpureum* only (Choi *et al.* 2009, Thines *et al.* 2009).

Apart from the sage pathogens reported so far, several other *Salvia* species were reported as hosts for *Peronospora*, such as *S. lanceolata*, *S. pratensis*, *S. reflexa* and *S. sclarea* (Rabenhorst 1857, Ellis & Kellerman 1887, Gäumann 1923, USDA 1960, Osipjan 1967, Kochman 1970, Stanjavičenie 1984). In checklists of *Peronosporaceae* in Europe and the British Isles downy mildews on *S. sclarea* (clary sage) and *S. pratensis* (meadow sage), have been noted in addition to downy mildew on common sage (Gaponenko 1972, Dudka *et al.* 2004, Mulenko *et al.* 2008, Müller & Kokes 2008). The downy mildews on clary and meadow sage were usually attributed to *Pe. swinglei* (Gaponenko 1972, Mulenko *et al.* 2008), or *Pe. lamii* (Preece 2002, Dudka *et al.* 2004, Müller & Kokes 2008), respectively. While *Pe. lamii* is clearly not an appropriate species name for downy mildews on sage (Choi *et al.* 2009, Thines *et al.*

2009), the application of the name *Pe. swinglei* to downy mildew pathogens of various species of sage seemed to be more plausible, because this taxon was originally described from *S. reflexa* (Ellis & Kellerman 1887, Constantinescu 1991). However, phylogenetic investigations revealed a very high degree of specialisation in *Peronospora* on *Lamiaceae* in general and on *Salvia* in particular (Choi *et al.* 2009, Thines *et al.* 2009). These studies demonstrated that *Pe. swinglei* was not only distinct from *Pe. belbahrii* but also from the two downy mildews infecting *S. officinalis* and *S. plebeia,* resulting in the description of *Pe. salviae-plebeiae* and *Peronospora salviae-officinalis* (Choi *et al.* 2009). Thus, three individual *Peronospora* taxa are currently reported from sages.

So far common sage is the only known host of *Pe. salviae-officinalis*. From a phytopathological perspective it is important to clarify whether other potential hosts do exist that could serve as reservoirs of inoculum for the disease caused by *Pe. salviae-officinalis*. Phylogenetic studies of *Lamiaceae* with a special focus on *Salvia* showed that *S. officinalis* and *S. sclarea* are closely related. Together with *S. pratensis*, they belong to the "Clade I" within the mint family (Walker & Sytsma 2007, Will & Classen-Bockhoff 2014). Because of their close phylogenetic relationship, it seemed possible that these sage species could be alternative hosts and could play a role in the infection of sage fields. At the same time, the downy mildew pathogen of coleus that also belongs to the *Pe. belbahrii* species complex seems still have a restricted distribution (Daughtrey *et al.* 2006, Palmateer *et al.* 2008, Denton *et al.* 2015, Ito *et al.* 2015, Gorayeb *et al.* 2019) suggesting that it is not conspecific with *Pe. belbahrii* and thus representing another downy mildew pathogen posing a potential economic risk.

It was the aim of the current study to better define species boundaries in the *Pe. belbahrii* species complex by detailed morphological and molecular phylogenetic investigations.

MATERIALS AND METHODS

Fungal specimens

The downy mildew specimens analysed in this study are given in Table 1.

Morphological analysis

The morphology of the investigated specimens was studied using a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) equipped with a Jenoptik ProgRes® digital camera. Nomarski Differential Interference Contrast (DIC) was used for observations, measurements and pictures. Images were taken using CapturePro v. 2.8 software (Jenoptik, Jena, Germany). Before measuring, herbarium specimens were moistened with 70 % alcohol and then transferred to 60 % lactic acid on a microscope slide. For all samples 100 conidia and conidiophores and 20 conidiophore stems were measured. All measurements are given in the form (minimum –) border of 30 % – mean – border of 30 % (– maximum) as suggested by Thines *et al.* (2009).

DNA extraction, PCR amplification, and sequencing

For DNA extraction about 1 mm² of infected plant tissue was excised using a sterile razor blade, transferred to a 2 mL reaction tube with three metal beads (3 mm diam, Qiagen), cooled down in liquid nitrogen and disrupted using a mixer mill (TissueLyser LT, Qiagen, Hilden, Germany) by shaking the tubes twice at 50 Hz for 90 s with an intervening cooling step. Genomic DNA was extracted using the innuPREP Plant DNA Kit (Analytik Jena, Jena, Germany). Four nuclear and two mitochondrial gene regions were amplified by PCR using newly designed or published primer pairs listed in Table 2. Initially amplification success was low for *ef1a*, *β-tubulin* and *hsp*90. Therefore, new primers were designed based on a draft genome of *Peronospora salviae-officinalis* (data not published). Amplification reactions were carried out in 25 µL including genomic DNA, 10 x Mango PCR Buffer, 1.5 U Mango Taq Polymerase (Bioline GmbH, Luckenwalde, Germany), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.4 µM forward and reverse primers. In cases where only weak PCR amplification was obtained, PCR was repeated using an ALLin Hot Start Taq Mastermix (HighQu GmbH, Kraichtal, Germany). PCR conditions were as follows: an initial denaturation step of 95 °C for 3 min, 40 cycles of 95 °C for 30 s, primer-specific annealing temperatures for 30 s (see Table 2), 72 °C extension for 90 s and final extension of 72°C for 10 min. PCR products were purified using a DNA Clean & Concentrator TM-5 Kit (Zymo Research Europe GmbH, Freiburg, Germany) and amplicons were sequenced at Eurofins Genomics (Eurofins Genomics GmbH, Ebersberg, Germany) using the primers that were used for PCR.

Phylogenetic analysis

In the phylogenetic analyses newly generated and already published sequences were used (see Table 1). The newly generated sequences were edited using the DNA Sequence Analysis Software Sequencher v. 5.4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). DNA sequences were aligned with the online version of MAFFT v. 7 (Katoh *et al.* 2017) using the iterative refinement algorithms Q-INS-i for the ITS rDNA and L-INS_i for all other gene regions. The start and end of the alignments were cut manually in Se-Al v. 2.0 (Rambaut 1996) to remove leading and trailing gaps. The final alignments obtained were deposited (www.treebase.org) and are available under accession no S25694 (http://purl.org/phylo/treebase/phylows/study/TB2:S25694).

Phylogenetic trees were inferred based on the alignments using maximum parsimony (MP), Bayesian Metropolis coupled Markov chain Monte Carlo analyses (MC^3), maximum likelihood (ML), and minimum evolution (ME). The MP and ME analysis were carried out in MEGA v. 7 (Kumar *et al.* 2016) using default settings. Support for internal nodes was estimated by 500 and 1 000 bootstrap replicates, respectively (Felsenstein 1985). The MC^3 analysis was performed using MrBayes v. 3.2 (Ronquist & Huelsenbeck 2003) applying GTR+I+G as the substitution model. For Bayesian analyses 1 M generations were run for the multi-locus tree and 2 M generations for the *cox*2-based tree, respectively, and trees were sampled every 500 generations. The 50 % majority rule consensus trees were computed and *a posteriori* probabilities (pp) estimated from trees of the plateau using a 20 % burnin. Maximum likelihood analyses were performed using RAxML v. 7.2.8 (Stamatakis 2014) as implemented in Geneious v. 8.1.2 (Biomatters Limited, Auckland, New Zealand) applying the general time-reversible (GTR) substitution model with gamma model of rate heterogeneity and 1 000 replicates of rapid bootstrapping. The phylogenetic trees were visualised within MEGA v. 7 or using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree).

Table 1. *Peronospora* specimens analysed in this study.

Pathogen	Host	Location	Year	Accession	Collector	ITS	*ef1a*	*ß-tub*	*Hsp*90	*cox*1	*cox*2
Pe. belbahrii	*Ocimum basilicum*	Germany, IGZ Großbeeren/ Erfurt	2017	FR-0162878	anonymous	MN308051	MN546882	MN546908	MN546985	MN546933	MN546959
Pe. belbahrii	*Ocimum basilicum*	Germany, IGZ Großbeeren/ Erfurt	2017	FR-0162878	anonymous	MN308052	MN546883	MN546909	MN546986	MN546934	MN546960
Pe. belbahrii	*Ocimum basilicum*	Germany, IGZ Großbeeren/ Erfurt	2017	FR-0162880	anonymous	MN308053	MN546884	MN546910	MN546987	MN546935	MN546961
Pe. belbahrii	*Ocimum basilicum*	Germany, NI, Braunschweig	2018	FR-0162881	M. Hoffmeister	MN450330	MN546899	MN546924	MN547000	MN546950	MN546976
Pe. belbahrii	*Ocimum basilicum*	Germany	2005	HOH HUH770	M. Thines	–	–	–	–	–	FJ394344*
Pe. belbahrii	*Ocimum basilicum*	Germany	2004	GLM74580	H. Jage	–	–	–	–	–	KJ654229*
Pe. elsholtziae	*Elsholzia ciliata*	Korea	2004	KUS-F20252	anonymous	MN450321	–	–	–	KJ654147*	KJ654296*
Pe. glechomae	*Glechoma hederacea*	Germany, ST, Östliches Harzvorland	2001	GLM-F73803	H. Jage	MN450323	MN546892	MN546919	MN546995	MN546943	KJ654217*
	Glechoma hederacea	Romania, Suceava, Clit	1992	BUCM 125.616	G. Negreen	MN450332	MN546901	MN546926	MN547002	MN546952	MN546978
Pe. lamii	*Lamium purpureum*	Germany, BW, Ladenburg	2018	FR-0162882	M. Hoffmeister	MN450324	MN546893	–	–	MN546944	MN546970
	Lamium purpureum	Germany, NI, Evessen	2018	FR-0162883	M. Hoffmeister	MN450325	MN546894	–	–	MN546945	MN546971
Pe. salviae-plebeiae	*Salvia plebeia*	Korea, Hongcheon	2008	KUS-F23371		–	–	–	–	–	KJ654299*
Pe. salviae-officinalis	*Salvia officinalis*	Germany, HE, Bad Hersfeld	2017	GLM-F117791	H. Blum	MN308035	MN546878	MN546904	MN546981	MN546929	MN546955
	Salvia officinalis	Germany, SN, Dresden	2017	GLM-F117792	C. Grunert	MN308036	MN546879	MN546905	MN546982	MN546930	MN546956
	Salvia officinalis	Switzerland, TG, Kesswil	2017	GLM-F117793	H. Blum	MN308034	MN546880	MN546906	MN546983	MN546931	MN546957
	Salvia officinalis	Germany, NI, Rittmarshausen	2017	GLM-F117794	M. Hoffmeister	MN450312	MN546881	MN546907	MN546984	MN546932	MN546958
	Salvia officinalis	Germany, RP, Worms	2016	GLM-F117795	M. Hoffmeister	MN450318	MN546889	MN546916	MN546992	MN547005	MN546967
Pe. saturejae-hortensis	*Satureja hortensis*	Germany	1996	GLM-F67681	H. Jage	–	–	–	–	KJ654094*	KJ654243*
Pe. choii	*Plectranthus scutellarioides*	USA, Michigan	2007	PsC3	C. Ehrhard	MN450320	MN546891	MN546918	MN546994	MN546942	MN546969
Pe. choii (Holotype)	*Plectranthus scutellarioides*	USA, Tenessee	2015	BPI 893223	A. Windham	MN450333	MN546902	MN546927	MN547003	MN546953	MN546979
Pe. choii (Paratype)	*Plectranthus scutellarioides*	USA, Tenessee	2015	BPI 893222	A. Windham	MN450334	MN546903	MN546928	MN547004	MN546954	MN546980

Table 1. (Continued).

Pathogen	Host	Location	Year	Accession	Collector	ITS	*ef1a*	*ß-tub*	*Hsp*90	*cox*1	*cox*2
	Plectranthus scutellarioides	USA	2007	HOH HUH945	anonymous	–	–	–	–	–	FJ394343*
	Plectranthus scutellarioides	USA	2007	HOH HUH946	anonymous	–	–	–	–	–	FJ394342*
	Plectranthus scutellarioides	USA	2007	HOH HUH947	anonymous	–	–	–	–	–	FJ394339*
	Plectranthus scutellarioides	USA	2007	HOH HUH948	anonymous	–	–	–	–	–	FJ394340*
	Plectranthus scutellarioides	USA, Tenessee	2015	BPI 893223	A. Windham	–	–	–	–	–	KT828759*
Pe. salviae-pratensis (Holotype)	*Salvia pratensis*	Germany, BW, Ladenburg	2016	GLM-F117783	M. Hoffmeister	MN450313	MN546885	MN546911	MN546988	MN546936	MN546962
Pe. salviae-pratensis (Paratype)	*Salvia pratensis*	Germany, NI, Braunschweig	2017	GLM-F117784	M. Hoffmeister	MN450314	MN546886	MN546912	MN546989	MN546937	MN546963
	Salvia pratensis	Germany, NI, Evessen	2017	GLM-F117785	M. Hoffmeister & W. Maier	MN450319	MN546890	MN546917	MN546993	MN546941	MN546968
	Salvia pratensis	Germany, RP, Mainz	2018	GLM-F117786	M. Hoffmeister	MN450326	MN546895	MN546920	MN546996	MN546946	MN546972
	Salvia pratensis	Germany, BW, Dossenwald	2018	GLM-F117787	M. Hoffmeister	MN450327	MN546896	MN546921	MN546997	MN546947	MN546973
	Salvia pratensis	Germany, BW, Nussloch	2018	GLM-F117788	M. Hoffmeister	MN450328	MN546897	MN546922	MN546998	MN546948	MN546974
	Salvia sclarea	Germany, BY, Schwebheim	2017	FR-0162877	M. Hoffmeister	MN450316	MN546887	MN546914	MN546990	MN546939	MN546965
	Salvia sclarea	Germany, NI, Braunschweig	2017	GLM-F117789	M. Hoffmeister	MN450317	MN546888	MN546915	MN546991	MN546940	MN546966
	Salvia sclarea	Germany, BB, Glindwo	2017	FR-0162876	V. Kummer	MN450331	MN546900	MN546925	MN547001	MN546951	MN546977
	Salvia sclarea	Germany, ST, Quedlinburg	2018	GLM-F117790	M. Hoffmeister	MN450329	MN546898	MN546923	MN546999	MN546949	MN546975
Pe. swinglei (Type)	*Salvia reflexa*	USA	1887	FH 00079723		–	–	–	–	–	FJ394338*
Pe. teucrii	*Teucrium botrys*	Germany	2004	GLM-F62880	V. Kummer	MN450322	–	–	–	KJ654108*	KJ654257*
Pe. viciae	*Vicia faba*	Germany, SN, Aschersleben	2017	FR-0162884	T. Kühne	MN450315	–	MN546913	–	MN546938	MN546964

* Sequences downloaded from GenBank.

Table 2. Primers used in this study.

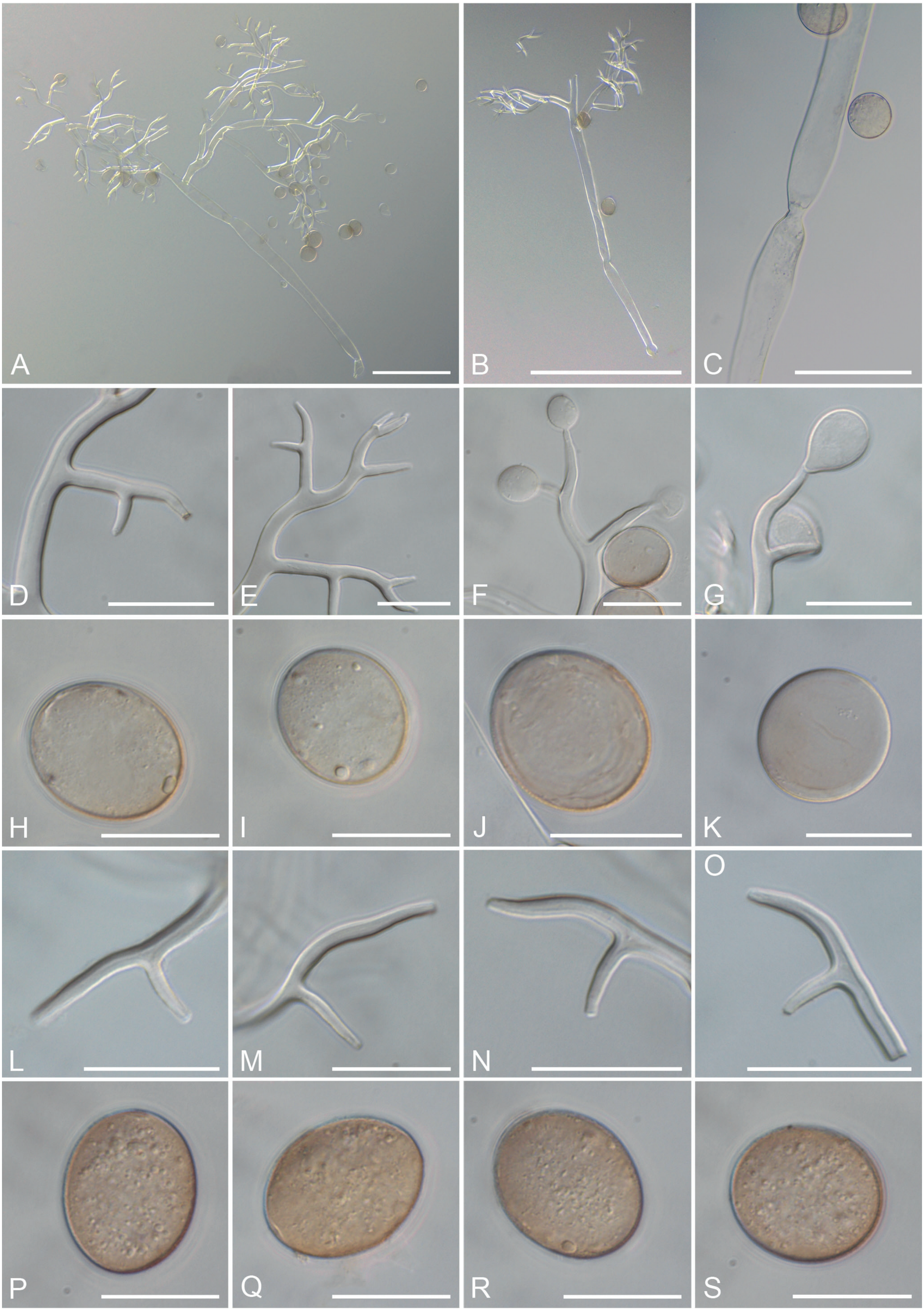
Locus	Primer	Sequence (5´ -> 3´)	T[b]	Reference
Nuclear				
ITS	DC6	GAGGGACTTTTGGGTAATCA	57	(Cooke 2000)
	LR-0	GCTTAAGTTCAGCGGGT		(Moncalvo 1995)
EF1a	EF1a Pso fd	ACATTGCCCTGTGGAAGTTCGA	61	This study
	EF1a Pso rv	AGTCTCAAGAATCTTACCCGAACGA		This study
ß-*tub*	bTub Pso fd	AATGAGGCTACAGGTGGACGTTA	58	This study
	bTub Pso rv	CACGCTTGAACATTTCTTGAATAGC		This study
hsp90	HSP90 Pso fd	GGTACTCATCGCTCACTGATG	54	This study
	HSP90 Pso rv	CAACGCCCTTTACAAATGACA		This study
Mitochondrial				
*cox*1	OomCox1-levup	TCAWCWMGATGGCTTTTTTCAAC	42	(Robideau *et al.* 2011)
	OomCox1-levlo	CYTCHGGRTGWCCRAAAAACCAAA		(Robideau *et al.* 2011)
*cox*2	cox2 forward	GGCAAATGGGTTTTCAAGATCC	42,5	(Hudspeth *et al.* 2000)
	cox2 reverse	CCATGATTAATACCACAAATTTCACTAC		(Hudspeth *et al.* 2000)

RESULTS

Morphology

The *Peronospora* species on *Pl. scutellarioides* (coleus) differs from *Pe. belbahrii* on *O. basilicum* (basil) in various aspects (Table 3 and Fig. 1). Conidia on *Pl. scutellarioides* were ellipsoid to rounded and with a pale brown colouration, whereas conidia of *Pe. belbahrii* were ovoid to long ellipsoid and with a dark brown to olive colouration. *Peronospora* on coleus further differed from *Pe. belbahrii* by a smaller conidial size: 19.9 × 18.7 µm in the former vs. 30.8 × 24.0 µm in the latter. Additionally, the mean length/width ratio from 1.13 to 1.16 of the former was smaller than that of the latter (mean = 1.29). The downy mildew on coleus differs from *Pe. belbahrii* also in the shape of the ultimate branchlets. The shape of ultimate branchlets in *Peronospora* on coleus was curved to almost straight, especially the shorter branch was often straight, while in *Pe. belbahrii* both were curved. In addition, the length of the ultimate branchlets and the ratio of the length of the longer to the shorter ultimate branchlet differed. The mean values of the longer branchlets of *Peronospora* on coleus were shorter (15.6 µm) than those of *Pe. belbahrii* (20.6 µm). With 9.2 and 9.8 µm, respectively (type and paratype) in the mean the shorter branchlets of *Peronospora* on coleus have a similar length as *Pe. belbahrii* (measuring 9.8 µm). As a consequence, the ratio of the length of the longer to the shorter ultimate branchlet was smaller for *Peronospora* on coleus (1.72) than that of *Pe. belbahrii* (2.29).

Conidial size and shape and conidiophore size and shape of the *Peronospora* species on *S. pratensis* were similar in all six sampling sites (measurements are only shown for two specimens, Table 4 and Fig. 2). The pathogen on *S. pratensis* differs from *Pe. swinglei* on *S. reflexa* and from *Pe. lamii* on *L. purpureum*. Conidia on *S. pratensis* were ovoid and showed a rounded base, whereas conidia of *Pe. swinglei* were often tear-shaped and narrowing/tapering at the base. Conidia of *Pe. lamii* were ovoidal to broadly ellipsoidal and often slightly narrowing at the base with a short pedicel. *Peronospora* on *S. pratensis* differed from *Pe. swinglei* and *Pe. lamii* by smaller conidial size: 21.0 × 18.3 µm in the former *vs*. 23.6 × 20.6 µm and 23.4 × 19.7 µm in the latter, respectively. Additionally, the mean length/width ratio from 1.15 of *P*. sp. on *Salvia pratensis* was smaller than that of *Pe. lamii* (mean = 1.19). Furthermore, conidia of *Peronospora* on *S. pratensis* differed from those of *Pe. glechomae* on *Glechoma hederacea*. With 22.6 × 17.2 µm and a mean length/width ratio of 1.31, conidia of *Pe. glechomae* were longer but narrower than those of *Peronospora* on *S. pratensis*. The conidial colour of *Peronospora* on *S. pratensis* was light greyish with a pale brownish hue whereas conidia from *Pe. glechomae* were vibrant brown. The ovoidal to ellipsoidal conidia of the *Peronospora* species on *S. pratensis* differed in their shape from the conidia of *Pe. salviae-officinalis,* which were ellipsoidal to broadly ellipsoidal. No differences were observed in mean conidial length, width and the mean length/width between these two species. The pathogen on *Salvia pratensis* differs from *Pe. swinglei* and *Pe. lamii* also in the shape of the ultimate branchlets. The shape of the ultimate branchlets in *Peronospora* on *S. pratensis* was slightly curved to almost straight, while in the latter two species it was straight or almost so. Also, the length of the ultimate branchlets and the ratio of the longer to the shorter ultimate branchlet differed. The longer branchlets of *Peronospora* on *S. pratensis* were longer (13.3 µm) than those of *Pe. swinglei* (11.6 µm) and *Pe. lamii* (12.3 µm), respectively. With 7.5 µm in the mean the shorter branchlets of *Peronospora* on *S. pratensis* were longer than those of *Pe. swinglei* (measuring 7.1 µm) but shorter than those of *Pe. lamii* (8.3 µm). Although the ultimate branchlets of the *Peronospora* species on *S. pratensis* and those of *Pe. salviae-officinalis* did not


A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S

differ significantly in morphometric measurements, they tended to be rather rounded in *Peronospora* on *S. pratensis* in contrast to subacute in *Peronospora salviae-officinalis*.

Conidia of *Peronospora* on *S. sclarea* are highly similar to those of *Pe. salviae-officinalis* on *S. officinalis*: They measure 21.5 × 18.4 µm in the former and 21.1 × 18.0 µm in the latter (type), and the 1.16 length/width ratio of the conidia was nearly the same as compared to the type of *Pe. salviae-officinalis* (mean = 1.17). The *s*hape and length of the ultimate branchlets and the ratio of the longer to the shorter ultimate branchlet of *Peronospora* on *S. sclarea*, were similar to those of *Pe. salviae-officinalis* (Table 4 and Fig. 3).

Phylogenetic analysis

Phylogenetic relationships inferred using MP, ME, ML and MC³ analyses based on the alignment of *cox*2 only are presented in Fig. 4, and the phylogenetic relationships calculated from the concatenated alignment of four nuclear (ITS, *ef*1a*, hsp*90*, β-tubulin*) and two mitochondrial (*cox*1*, cox*2) loci are presented in Fig. 5. The *cox*2-only alignment had 419 characters. The concatenated alignment comprised 4 049 characters: *i.e. cox*1 (527), *cox*2 (487), ITS (927), *ef*1*a* (677), *hsp*90 (804) and *β-tubulin* (627). Since no conflicts in supported groupings were found between the tree topologies of the MP, ME, ML and MC³ analyses, only the topology of the MP tree is shown for the *cox*2 analysis in Fig. 4 and for the multi loci analysis in Fig. 5, with addition of the support values of the other analyses. Two most parsimonious trees were found in the MP analysis of the *cox*2-data set and six in the combined data set, respectively with minor differences in the topology of unsupported groupings. One of these trees each was selected for presentation.

The single gene analysis based on *cox*2 sequences showed sufficient resolution to distinguish between *Peronospora* from coleus, basil, and the different sage species (Fig. 4), respectively (except for the pathogens on clary and common sage). It also again showed that *Pe. lamii s. str*. on *Lamium purpureum* and *Pe. swinglei s. str*. on *Salvia reflexa* are only distantly related to each other (compare Choi *et al.* 2009, Thines *et al.* 2009) and to the here newly sampled downy mildews on clary and meadow sage. The combined six-gene analysis showed a more resolved and better supported tree topology (Fig. 5). The monophyly of lineages parasitic to specific host species received mostly high to maximum support values in the multi gene analyses, except for the two specimens of *Pe. glechomae*, which did not receive any significant support in the analyses. The downy mildew pathogens of meadow sage formed two distinct and each well-supported clades that grouped together with moderate to strong support. The monophyly of *Pe. belbahrii* and coleus downy mildew pathogens, respectively, received maximum support in all analyses in the phylogenetic tree based on six loci. In contrast to *Pe. belbahrii* on basil*,* which showed intraspecific variability, the downy mildews specimens from coleus were identical in all six gene regions studied. The downy mildew pathogens on common and clary sage grouped together with mostly strong support in both the *cox*2 and in the reconstruction based on six loci.

Taxonomy

Due to differences in morphology and on the basis of molecular phylogenetic reconstructions, it is concluded that the *Peronospora* specimens studied from *Pl. scutellarioides* and *S. pratensis* are sufficiently distinct from other *Peronospora* species on *Mentheae* to propose them as new species.

Peronospora choii Hoffmeister, W. Maier & Thines, ***sp. nov.*** MycoBank MB834424. Fig. 1A–K.

Etymology: The species is dedicated to Young-Joon Choi for his significant contributions to the phylogeny and taxonomy of downy mildews.

Typus: **USA**, Tennessee, on living leaves of *Plectranthus scutellarioides*, Aug. 2015, *A. Windham* (**holotype** BPI 893223).

Habitat: On living leaves of *Plectranthus scutellarioides* (syn.: *Solenostemon scutellarioides*, *Coleus scutellarioides*; *Lamiaceae*).

Straminipila, Peronosporomycetes, Peronosporales, Peronosporaceae. *Hyphae* intercellular, *haustoria* intracellular. *Down* dainty floccose, greyish to brownish. *Conidiophores* emerging from stomata, hyaline, slender, length 351–831 µm; trunk erect, straight or slightly curved, 222–533 µm long, 10–21 µm broad below the first branch, basal end often slightly swollen, 8–14 µm broad, sometimes constricted at middle height, callose plugs not observed; branching submonopodial, branched 4–6(–7) times, branches slightly curved, arborescent. *Ultimate branchlets* slightly curved to almost straight, obtuse, in pairs with different lengths, the longer being usually (8.2–) 12.7–15.6–17.2(–27.4) µm long, the shorter (4.2–)7.9–9.2–10.3(–14.9) µm, longer/shorter branch ratio (1.01–)1.53–1.72–1.83(–3.12). *Conidia* light greyish to pale brownish, ovoidal to ellipsoidal, (14.6–)17.9–19.9–21.4(–27.3) µm long, (12.8–)15.9–17.8–18.9(–24.9) µm broad, length/breadth ratio (1.02–)1.08–1.13–1.16(–1.33), tip and base rounded; wall ornamentation obscure; pedicel absent. *Oospores* not seen.

Additional material examined: **USA**, Tennessee, on living leaves of *Plectranthus scutellarioides*, Aug. 2015, *A. Windham* (paratype BPI 893222).

Notes: Infected leaves show discoloured, chlorotic to necrotic spots as seen from the upper surface. On the lower surface of the leaves a grey to brown down of conidiophores with conidia is formed in the lesions.

Peronospora salviae-pratensis Hoffmeister, W. Maier & Thines, ***sp. nov.*** MycoBank MB834425. Fig. 2A–I.

Etymology: *"salviae-pratensis*"refers to the Latin species name of the host plant.

Fig. 1. A–K. *Peronospora choii* on *Plectranthus scutellarioides* (BPI 893223). **L–S.** *Peronospora belbahrii* on *Ocimum basilicum*. **A-C.** Conidiophores. **D, E, L–O**. Ultimate branchlets of conidiophores. **F, G**. Ultimate branchlets of conidiophores with developing conidia. **H–K, P–S**. Mature conidia. Scale bars: A = 100 µm; B, C = 50 µm; D–S = 20 µm.

Table 3. Comparison of morphological features of *Peronospora* spp. parasitic to coleus, basil and red deadnettle.

Pathogen	***Pe. plectranthi***	***Pe. plectranthi***	***Pe. plectranthi***	***Pe. belbahrii***	***Pe. belbahrii***	***Pe. lamii***
Host	*Plectranthus scutellarioides*	*Plectranthus scutellarioides*	*Plectranthus scutellarioides*	*Ocimum basilicum*	*Ocimum basilicum*	*Lamium purpureum*
Acc. no.	BPI 893223 (Type)	BPI 893222 (Paratype)	HOH, HUH 946	HOH, HUH 770 (Type)	FR-0162878	FR-0162882
Ultimate branchlets						
Shape	Curved to sub-straight	Curved to sub-straight	Curved	Curved	Curved	Sub-straight
Length (longer)	(8.2–)12.7–15.6–17.2(–27.4) µm	(8.0–)14.3–17.6–18.8(–36.5) µm	(6.4–)10.0–13.4–17.0(–26.0) µm	(13.0–)18.0–20.6–26.0(–31.0) µm	(8.9–)16.2–18.0–19.9(–28.2) µm	(6.1–)10.6–12.3–14.3(–18.6) µm
Length (shorter)	(4.2–)7.9–9.2–10.3(–14.9) µm	(4.2–)7.7–9.8–10.9(–25.7) µm	(5.1–)5.4–7.7–8.9(–15.0) µm	(3.8–)7.7–9.8–10.0(–15.0) µm	(5.2–)7.5–9.1–10.5(–14.7) µm	(5.0–)6.9–8.3–9.5(–13.6) µm
Longer/shorter ratio	(1.01–)1.53–1.72–1.83(–3.12)	(0.96–)1.69–1.84–1.96(–2.75)	(1.3–)1.6–1.88–2.2(–3.5)	(1.30–)1.80–2.29–2.70(–4.00)	(1.28–)1.88–2.00–2.12(–3.18)	(1.05–)1.36–1.50–1.59(–2.44)
Shape of tips of ultimate branchlets	Acute to subacute, sometimes rounded	Acute to subacute, sometimes rounded	Acute to subacute	Acute to subacute	Acute to subacute	Obtuse to subacute
Conidia						
Shape	Ellipsoid to rounded	Ellipsoid to rounded	Ellipsoid	Ovoid	Ovoid	Ovoid to broadly ellipsoid
Colour	Light brown	Light Brown	Brown	Dark brown to olive	Dark brown to olive	Light brownish to greyish
Base	Rounded	Rounded	Often rounded, sometimes narrowed	Rounded	Rounded	Rounded
Length	(14.6–)17.9–19.9–21.4(–27.3) µm	(20.6–)24.0–25.3–26.5(–31.7) µm	(22.0–)24.0–26.3–28.0(–32.0) µm	(24.0–)29.0–30.8–33.0(–36.0) µm	(22.6–)25.9–26.9–27.9(–30.5) µm	(20.3–)22.3–23.4–24.0(–27.3) µm
Width	(12.8–)15.9–17.8–18.9(–24.9) µm	(18.5–)20.9–22.0–22.8(–27.3) µm	(18.0–)20.0–21.3–23.0(–24.0) µm	(20.0–)23.0–24.0–26.0(–29.0) µm	(18.4–)21.9–22.5–23.2(–26.1) µm	(17.0–)19.1–19.7–20.3(–22.6) µm
Length/width ratio	(1.02–)1.08–1.13–1.16(–1.33)	(1.06–)1.12–1.16–1.18(–1.33)	(1.1–)1.2–1.24–1.3(–1.5)	(1.10–)1.20–1.29–1.40(–1.50)	(1.05–)1.16–1.19–1.23(–1.35)	(1.06–)1.16–1.19–1.21(–1.33)
Pedicel	Absent	Absent	Absent	Absent	Absent	Mostly absent, rarely with a scar
Wall ornamentation	Obscure	Obscure			Obscure	Obscure
Haustoria						
Shape	Not seen	Not seen	Not seen (Thines *et al.* 2009)	Not seen (Thines *et al.* 2009)	Pyriform to globose	Pyriform to globose

Table 4. Comparison of morphological features of *Peronospora* spp. parasitic to *Salvia* spp. and *Glechoma hederacea*.

Fungus	***Pe. choii***	***Pe. choii***	***Pe. salviae-officinalis***	***Pe. salviae-officinalis***	***Pe. salviae-officinalis***	***Pe. swinglei***	***Pe. glechomae***
Host	*Salvia pratensis*	*Salvia pratensis*	*Salvia sclarea*	*Salvia officinalis*	*Salvia officinalis*	*Salvia reflexa*	*Glechoma hederacea*
Acc. no.	GLM-F117784 (Type)	GLM-F117783 (Paratype)	GLM-F117789	GLM-F117795	HOH: HUH 961 (Type)	FH 00079723 (Type)	GLM 73803
Ultimate branchlets							
Shape	Slightly curved to sub-straight	Slightly curved to sub-straight	Slightly curved to sub-straight	Slightly curved to sub-straight	Slightly curved to sub-straight	Sub-straight to straight	Slightly curved
Length (longer)	(7.5–)11.8–13.3–14.7(–22.1) µm	(7.2–)11.9–14.0–15.3(–25.2) µm	(8.8–)12.1–14.0–15.8(–21.4) µm	(7.3–)12.2–14.2–15.6(–22.2) µm	(8.0–)10.3–12.7–15.0(–17.5) µm	(8.8–)9.9–11.6–13.4(–15.5) µm	(7.9–)12.1–13.8–15.2(–22.6) µm
Length (shorter)	(4.4–)6.5–7.5–8.4(–11.7) µm	(4.9–)7.3–8.3–8.9(–14.8) µm	(4.9–)6.8–7.9–8.8(–12.8) µm	(4.1–)7.0–8.0–8.8(–13.0) µm	(5.0–)6.2–7.8–9.4(–10.0) µm	(4.5–)5.7–7.1–8.6(–10.0) µm	(4.9–)6.7–7.6–8.3(–11.5) µm
Longer/shorter ratio	(1.18–)1.63–1.78–1.91(–2.66)	(1.11–)1.57–1.71–1.81(–2.83)	(1.22–)1.67–1.81–1.91(–2.53)	(1.25–)1.63–1.80–1.92(–2.69)	(1.25–)1.33–1.66–1.99(–2.50)	(1.18–)1.4–1.67–1.93(–2.22)	(1.32–)1.64–1.83–1.97(–2.67)
Shape of tips of ultimate branchlets	Rounded to subacute	Rounded to subacute	Obtuse to subacute	Subacute	Subacute	Obtuse to subacute	Pointed and rounded, sometimes acute
Conidia							
Shape	Ovoid to ellipsoid	Ovoid to ellipsoid	Ellipsoid to broadly ellipsoid	Ellipsoid to broadly ellipsoid	Ellipsoid to broadly ellipsoid	Broadly ellipsoid to tear-shaped	Broadly ellipsoid
Colour	Light greyish to pale brownish	Light greyish to pale brownish	Light brownish to greyish	Light brownish to greyish	Light brownish to greyish	Brownish	Brownish
Base	Rounded	Rounded	Rounded	Rounded	Rounded	Often narrowing	Rounded
Length	(18.3–)20.2–21.0–21.5(–25.3) µm	(18.0–)21.6–22.3–23.6(–25.7) µm	(17.8–)20.6–21.5–22.4(–26.6) µm	(17.5–)19.4–19.9–20.4(–23.1) µm	(18.0–)19.5–21.1–22.7(–25.0) µm	(20.0–)22.0–23.6–25.3(–27.5) µm	(19.0–)21.8–22.6–23.4(–26.0) µm
Width	(15.7–)17.8–18.3–18.6(–21.8) µm	(15.1–)18.5–19.2–20.0(–22.2) µm	(16.0–)17.6–18.4–19.2(–21.6) µm	(15.3–)16.9–17.4–17.7(–19.7) µm	(16.3–)16.8–18.0–19.1(–22.3) µm	(17.5–)19.2–20.6–21.9(–22.5) µm	(13.9–)16.5–17.2–18.0(–20.4) µm
Length/width ratio	(1.04–)1.11–1.15–1.17(–1.25)	(1.05–)1.14–1.16–1.19(–1.25)	(1.04–)1.13–1.16–1.19(–1.28)	(1.06–)1.12–1.15–1.17(–1.25)	(1.04–)1.10–1.17–1.25(–1.37)	(1.02–)1.10–1.15–1.20(–1.25)	(1.24–)1.26–1.31–1.34(–1.46)
Pedicel	Absent	Absent	Absent	Absent	Absent	Absent, rarely with a scar	Absent
Wall ornamentation	Obscure	Obscure	Obscure	Obscure	Obscure	Prominent	Obscure
Haustoria							
Shape	Ellipsoid-pyriform	Ellipsoid-pyriform	Not seen	Ellipsoid-pyriform to globose	Globose to lobate	Not seen	Not seen
					(Thines *et al.* 2009)	(Thines *et al.* 2009)	

All measurements given in the form (minimum–) border of 30 %– mean – border of 30 %(maximum).

Typus: **Germany**, Baden-Wuerttemberg, Ladenburg, Waldpark (49°28’15.2’’N 8°37’04.2E), on living leaves of *Salvia pratensis*, 30 Apr. 2016, *M. Hoffmeister* (**holotype** GLM-F117783).

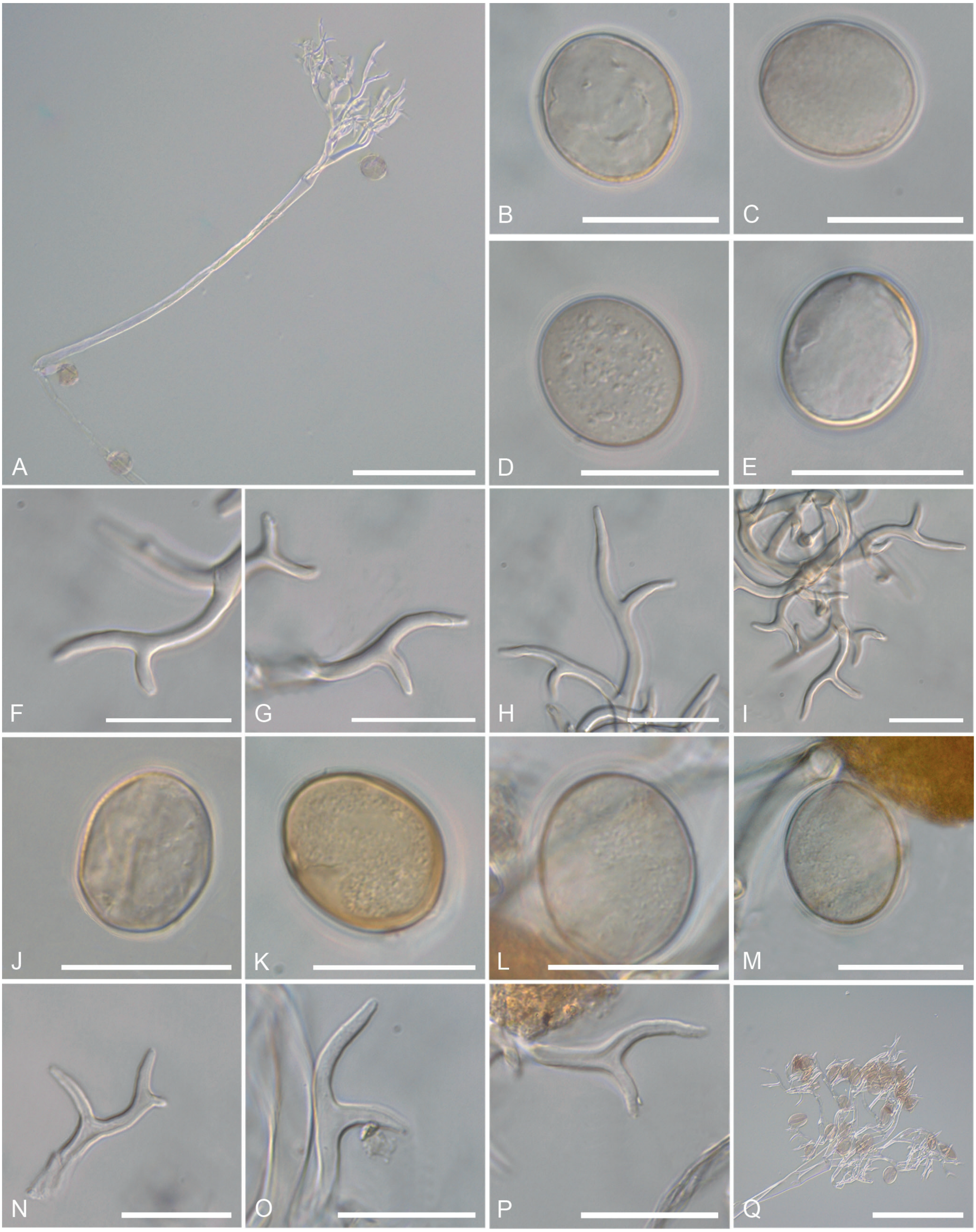


Fig. 2. A–I. *Peronospora salvia-pratensis* on *Salvia pratensis*. **J-Q.** *Peronospora glechomae* on *Glechoma hederacea*. **A, Q.** Conidiophore. **B–E, J–M.** Conidia. **F–I, N–P.** Ultimate branchlets. Scale bars: A = 200 µm; B–P = 20 µm; Q = 100 µm.

Habitat: On living leaves of *Salvia pratensis* (*Lamiaceae*).

Straminipila, Peronosporomycetes, Peronosporales, Peronosporaceae. *Hyphae* intercellular, *haustoria* intracellular, mostly limited to one haustorium per host cell, lobate to globose. *Down* dainty floccose, whitish to cream. *Conidiophores* emerging from stomata, hyaline, slender, length overall 185–541 µm; trunk erect, straight or slightly curved, 85–380 µm long, 8–14 µm wide below the first branch, basal end not differentiated to slightly swollen, 7–12 µm wide at the base, callose plugs absent; branching monopodial to subdichotomous, branched 4–6(–7) times, branches slightly curved, arborescent. *Ultimate branchlets* slightly curved to almost straight, obtuse, in pairs with different lengths, the longer being usually (7.5–)11.8–13.3–14.7(–22.1) µm long, the shorter (4.4–)6.5–7.5–8.4(–11.7) µm, longer/shorter branch ratio (1.18–)1.63–1.78–1.91(–2.66). *Conidia* light greyish to pale brownish, ovoidal to ellipsoidal, (18.3–)20.2–21.0–21.5(–25.3) µm long, (15.7–)17.8–18.3–18.6(–21.8) µm broad, length/breadth ratio (1.04–)1.11–1.15–1.17(–1.25), tip and base rounded; wall ornamentation obscure; pedicel absent. *Oospores* not seen.

Fig. 3. A–G. *Peronospora salviae-officinalis* on *Salvia sclarea*. **H–K.** *Peronospora salviae-officinalis* on *S. officinalis*. **L–O.** *Peronospora lamii* on *Lamium purpureum*. **A, E, H.** Conidiophores. **B–D, J–N.** Conidia. **F, G, I, O.** Ultimate branchlets. Scale bars: A = 200 µm; B–D, E, G, I–O = 20 µm; E = 50 µm; H = 100 µm.

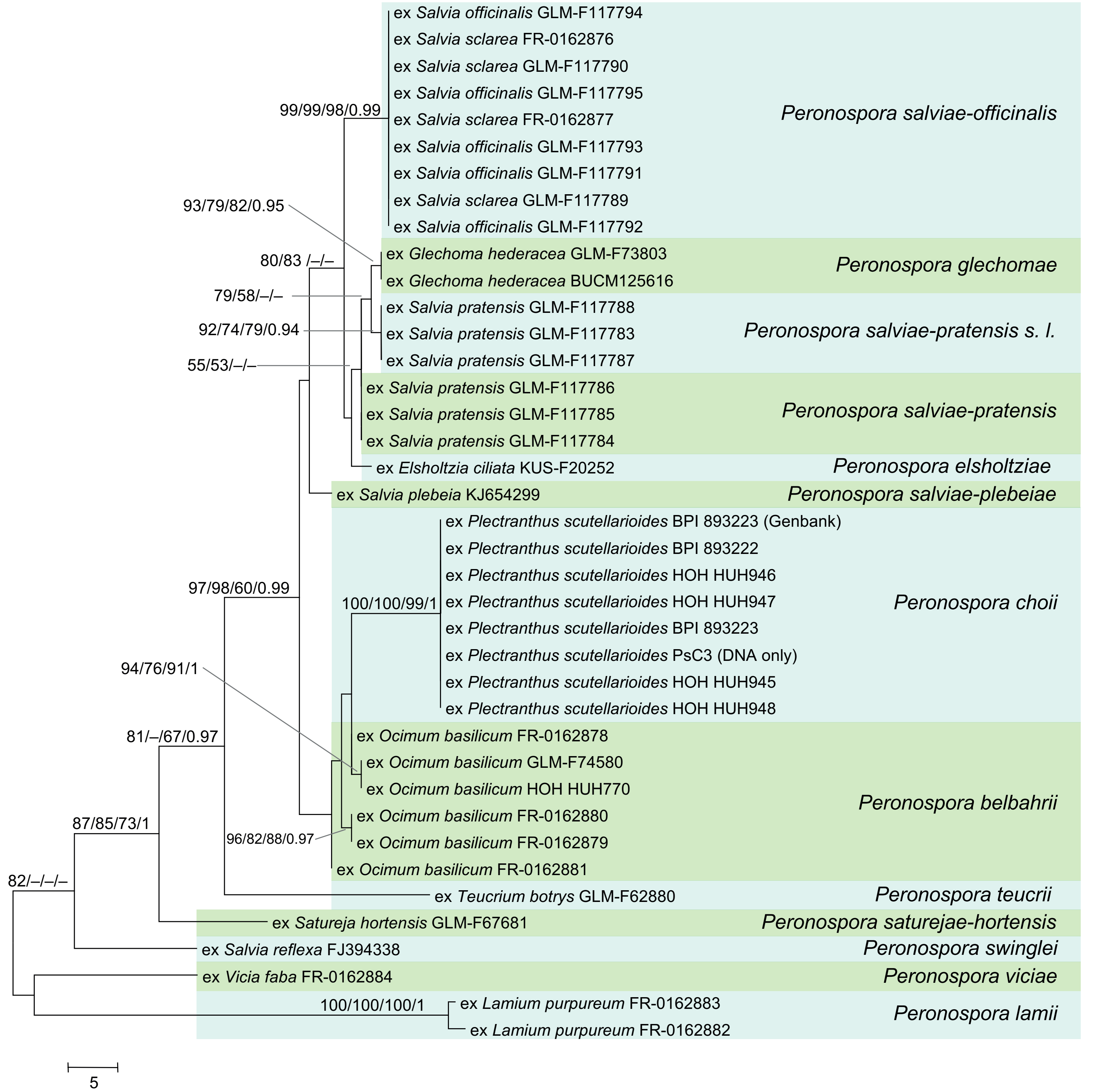


Fig. 4. Phylogenetic reconstruction in MP inferred from a *cox*2 alignment. MP, ME and ML bootstrap support values above 50 %, and *a posteriori* probabilities above 0.9 are given at first, second, third, and fourth positions at the branches, respectively. A minus sign denotes lacking support for the present or an alternate topology. No conflicting support was observed.

Additional materials examined: **Germany**, Lower Saxony, Evessen, quarry (52°11'53.9"N 10°43'19.8"E), 21 Jul. 2017, *M. Hoffmeister & W. Maier* (GLM-F117785); Rhineland-Palatinate, Mainz, Botanical Garden (49°59'28.6"N 8°14'27.8"), 27 Apr. 2018 (DE-O-MJG-200809901/1), *M. Hoffmeister* (GLM-F117786); Lower Saxony, Braunschweig (52°16'32.2"N; 10°34'04.1"E), 2 May 2018, *M. Hoffmeister* (GLM-F117784); Baden-Württemberg, Mannheim, Dossenwald (49°26'34.8"N 8°32'29.9"E), 10 May 2018, *M. Hoffmeister* (GLM-F117787); Baden-Wuerttemberg, Nussloch, meadow near quarry (49°19'01.2"N 8°43'01.5"), 13 May 2018, *M. Hoffmeister* (GLM-F117788).

Notes: Infected leaves show discoloured, yellowed and chlorotic to necrotic, polyangular, clearly vein-limited spots, as seen from the upper surface. On the lower surface of the leaves a pale brown down of conidiophores with conidia is formed in the lesions, which darkens with age.

DISCUSSION

Even though more than 400 species have already been described in *Peronosporaceae*, the vast majority of species in this genus remains to be discovered (Thines & Choi 2016). Especially the

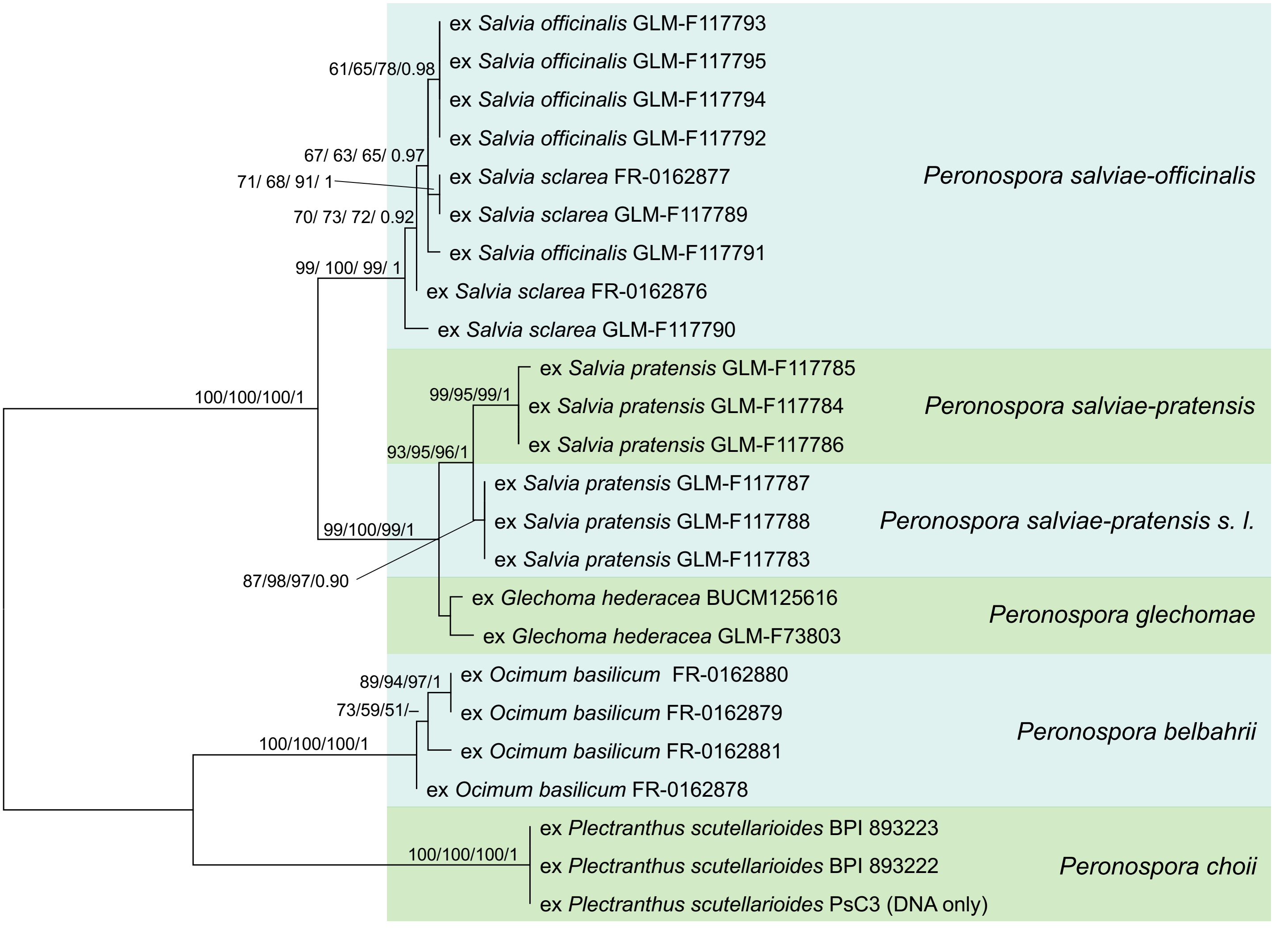


Fig. 5. Phylogenetic reconstruction in MP inferred from the concatenated alignment of six genes (ITS, *ef1a, hsp90, βtub*, *cox*1, *cox*2). MP, ME and ML bootstrap support values above 50 %, and *a posteriori* probabilities above 0.9 are given at first, second, third, and fourth positions at the branches, respectively. A minus sign denotes lacking support for the present or an alternate topology.

downy mildews of *Fabaceae* (Garcia-Blazquez *et al.* 2008) and *Amaranthaceae* (Choi *et al.* 2015a) seem to be highly diverse, but also for the *Lamiaceae*, several dozens of hosts have been reported (Constantinescu 1991, Dick 2001). Considering the high degree of host specialisation of members of the genus *Peronospora* (Thines & Choi 2016), it seems likely that this family harbours several undescribed downy mildew agents. Within *Lamiaceae*, the tribe *Mentheae* contains several *Peronospora* species occurring on culinary herbs and medicinal plants (Dick 2001). Two species belonging to the *Peronospora belbahrii* species complex, *Pe. belbahrii* and *Pe. salviae-officinalis*, have proven to be particularly destructive as emerging pathogens in basil and common sage production, respectively. In this study phylogenetic analyses of downy mildews on *Lamiaceae* were performed using six loci. The combined use of nuclear and mitochondrial gene regions resulted in generally highly-resolved clades and no supported discordance between mitochondrial and nuclear loci was observed, which is in line with previous studies (Choi & Thines 2015, Choi *et al.* 2015a), and in contrast to the findings of a recent study on *Peronosporaceae* (Bourret *et al.* 2018). As previously shown, ITS data were highly similar for closely related species of *Peronospora* (Thines *et al.* 2009, Voglmayr *et al.* 2014, Choi *et al.* 2015b). In contrast, *cox*2 resolved most of the lineages that were found by the six-gene phylogeny and thus qualified as a suitable barcoding marker for *Peronospora* species (Choi *et al.* 2015b). In addition, DNA extracted from older fungarium samples can be successfully used for amplification of the *cox*2 gene (Telle & Thines 2008, Choi *et al.* 2015b). The *cox*1, *ef*1a, *hsp*90 and *β-tubulin* genes also performed well in terms of phylogenetic resolution, and, after primer optimisation (Table 2), could also be amplified reliably.

In the present study, it was shown that the *Peronospora* species on *Pl. scutellarioides* and *Pe. belbahrii* can be reliably distinguished by differences in conidial shape, size and colouration, as well as by the shape of the ultimate branchlets of the conidiophores. In addition, phylogenetic analyses using four nuclear and two mitochondrial gene regions clearly resolved the downy mildew affecting *Pl. scutellarioides* as a highly supported monophyletic group, and, thus, it is described as *Pe. choii* in this study. The downy mildew disease of coleus had initially been lumped within *Pe. lamii* (Daughtrey *et al.* 2006, Palmateer *et al.* 2008), but was then relegated to *Peronospora belbahrii s. l.* (Thines *et al.* 2009). In that study it was already suggested that it might represent a species of its own, which is confirmed by the present study. It can therefore be assumed that in nature coleus downy mildew does not serve as inoculum source for basil downy mildew and *vice versa* although limited artificial infection of basil by *Pe. choii* had been demonstrated (Palmateer *et al.* 2008). This is in line with infection studies of other downy mildews in which broader potential host ranges than commonly present in nature could be observed under laboratory conditions (*e.g.* Runge & Thines 2008, Runge *et al.* 2012). Considering this and the fact that *Pe. choii* was so far only reported for Japan (Ito *et al.* 2015), UK (Denton *et al.* 2015), the USA (Daughtrey *et al.* 2006, Palmateer *et al.* 2008), and recently from Brazil (Gorayeb *et al.* 2019), but it is not yet as widely distributed as *Pe. belbahrii*, quarantine measures might still be useful to prevent the further spread of this disease throughout the world.

Based on phylogenetic inferences the downy mildews parasitizing *S. sclarea* and *S. pratensis*, respectively, were clearly distinct from *Pe. lamii,* but also from *Pe. swinglei.* Phylogenetic as well as the morphological investigations strongly support that the downy mildew on *S. sclarea* is conspecific with *Pe. salviae-officinalis*, thus this host has to be added to the host range of this species.

Salvia sclarea and *S. officinalis* are closely related and also have an overlapping natural geographical distribution. Whether one of the two host species was initially colonised by a host jump from the other host can only be speculated at this stage.

From a phytopathological point of view, the results from this study showed that the wild sage species *S. pratensis* most likely does not play any role as primary inoculum for downy mildew epidemics in cultivated common sage as it only seems to host a specific downy mildew species. In contrast, clary sage, which is closely related to common sage and is also cultivated as a medicinal plant, likely acts as alternative host for *Pe. salviae-officinalis* and is a potential inoculum source for the dissemination of this disease.

The *Peronospora* accessions from *S. pratensis* are very closely related to *Pe. glechomae* and together they form a sister group to *Peronospora salviae-officinalis*. The morphological and molecular phylogenetic differences between the samples of *Peronospora* found on meadow sage and those from *Pe. glechomae* are subtle. Nevertheless, is seems justified to consider the downy mildew on *S. pratensis* as a species of its own, *Pe. salviae-pratensis*, and not as conspecific with *Pe. glechomae*, described from *Glechoma hederacea* (Oescu & Radulescu 1939). Interestingly, *Pe. glechomae* was reported only a few times since it was first described as a new species from Romania (Oescu & Radulescu 1939, Müller & Kokes 2008). Despite significant efforts we could not find *Pe. glechomae* over a period of three years, whereas downy mildew on *S. pratensis* was easily found at different locations in Germany where *S. pratensis* populations were screened. This is in line with the numerous reports of downy mildew on meadow sage by other authors (Gaponenko 1972, Preece 2002, Dudka *et al.* 2004, Brandenburger & Hagedorn 2006, Mulenko *et al.* 2008, Müller & Kokes 2008). In contrast to the rare observations of downy mildew on ground ivy, the host plant itself is a very frequent perennial *Lamiaceae*, naturally distributed over large parts of Europe and west-northern Asia, and has also been introduced into North America (Meusel 1994). Considering that the sister group-relationship of the other sage-downy mildew accessions included in the multilocus analyses received maximum support in all analyses, it could be speculated that *Pe. glechomae* in fact is an incidental host and the few collections resulted from accidental observations of rare host jumps of a downy mildew species originating from meadow sage, for which the original host has not been included in molecular phylogenies, so far. It is also noteworthy that *Pe. salviae-pratensis* accessions from *S. pratensis* formed two distinct clades. It will be interesting to see, if with the addition of more specimens from *S. pratensis* this separation would still be found, suggesting independently evolved populations now both being present in Europe, similar to the situation in *Pseudoperonospora cubensis* (Runge *et al.* 2011), or if intermediate lineages will be observed, which would be suggestive of a diversified gene pool, similar to the situation observed in *Albugo candida* (Ploch *et al.* 2010). In any case, it seems that the *Pe. belbahrii* species complex is still in the phase of active radiation, rendering the discovery of new hosts for some of the species likely, especially if outside their native ranges (Thines 2019).

ACKNOWLEDGEMENTS

This study was funded by grants from the Federal Ministry of Food and Agriculture by decision of the German Bundestag. We thank Petra Westphal for technical assistance. The curators of the U.S. National Fungus Collection of the USDA, the Botanical Garden Braunschweig, the Botanical Garden Mainz and the Leibniz-Institute of vegetable and ornamental plants Großbeeren/Erfurt (IGZ), Hanna Blum, and Volker Kummer are gratefully acknowledged for providing specimens. Markus Scholler is also thanked for providing a specimen, which was not included in the present study due to contamination by a cultivable oomycete.

Conflict of interest: The authors declare that there is no conflict of interest.

REFERENCES

Belbahri L, Calmin G, Pawlowski J, *et al.* (2005). Phylogenetic analysis and real time PCR detection of a presumbably undescribed *Peronospora* species on sweet basil and sage. *Mycological Research* **109**: 1276–1287.

Bourret TB, Choudhury RA, Mehl HK, *et al.* (2018). Multiple origins of downy mildews and mito-nuclear discordance within the paraphyletic genus *Phytophthora*. *PLoS One* **13**: 1–23.

Brandenburger W, Hagedorn G (2006). Zur Verbreitung von *Peronosporales* (inkl. *Albugo*, ohne *Phytophthora*) in Deutschland. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* **405**: 1–174.

Choi Y-J, Klosterman SJ, Kummer V, *et al.* (2015a). Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (*Straminipila, Oomycota*), including pathogens of beet and spinach. *Molecular Phylogenetics and Evolution* **86**: 24–34.

Choi YJ, Beakes G, Glockling S, *et al.* (2015b). Towards a universal barcode of oomycetes - a comparison of the *cox*1 and *cox*2 loci. *Molecular Ecology Resources* **15**: 1275–1288.

Choi YJ, Shin HD, Thines M (2009). Two novel *Peronospora* species are associated with recent reports of downy mildew on sages. *Mycological Research* **113**: 1340–1350.

Choi Y-J, Thines M (2015). Host jumps and radiation, not co-divergence drives diversification of obligate pathogens. A case study in downy mildews and *Asteraceae*. *PLoS One* **10**: e0133655.

Constantinescu O (1991). An annotated list of *Peronospora* names. *Thunbergia* **15**: 1–110.

Daughtrey ML, Holcomb GE, Eshenaur B, *et al.* (2006). First report of downy mildew on greenhouse and landscape coleus caused by a *Peronospora* sp. in Louisiana and New York. *Plant Disease* **90**: 1111.

Denton GJ, Beal E, Denton JO, *et al.* (2015). First record of downy mildew, caused by *Peronospora belbahrii*, on *Solenostemon scutellarioides* in the UK. *New Disease Reports* **31**: 14.

Dick MW (2001). *Straminipilous fungi: Systematics of the peronosporomycetes including accounts of the marine straminipilous protists, the plasmodiophorids and similar organisms*. Kluwer, Netherlands.

Dudka IO, Heluta VP, Tykhonenko YY, *et al.* (2004). *Fungi of the Crimean Peninsula*. M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Ukraine.

Ellis JB, Kellerman WA (1887). New Kansas Fungi. *The Journal of Mycology* **3**: 102–105.

Felsenstein J (1985). Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution* **39**: 783–791.

Gamliel A, Yarden O (1998). Diversification of diseases affecting herb crops in Israel accompanies the increase in herb crop production. *Phytoparasitica* **26**: 53–58.

Gaponenko NI (1972). *The family Peronosporaceae of Middle Asia and south Kazakhstan: a classification key*. Institute of the Botanical Academy of Science of the Uzbek SSR, Uzbekistan.

Garcia-Blazquez G, Goker M, Voglmayr H, *et al.* (2008). Phylogeny of *Peronospora*, parasitic on *Fabaceae*, based on ITS sequences. *Mycological Research* **112**: 502–512.

Gäumann E (1923). *Beiträge zu einer Monographie der Gattung Peronospora Corda*. Zürich, Switzerland.

Gorayeb ES, Pieroni LP, Cruciol GCD, *et al.* (2019). First report of downy mildew on coleus (*Plectranthus* spp.) caused by *Peronospora belbahrii sensu lato* in Brazil. *Plant Disease* **104**: 294.

Hill CF, Pearson HG, Gill GSC (2004). *Peronospora dianthi* and *Peronospora lamii*, two downy mildews recently detected in New Zealand. *New Zealand Plant Protection* **57**: 348

Humphreys-Jones DR, Barnes AV, Lane CR (2008). First report of the downy mildew *Peronospora lamii* on *Salvia officinalis* and *Rosmarinus officinalis* in the UK. *Plant Pathology* **57**: 372.

Ito Y, Takeuchi T, Matsushita Y, *et al.* (2015). Downy mildew of coleus caused by *Peronospora belbahrii* in Japan. *Journal of General Plant Pathology* **81**: 328–330.

Katoh K, Rozewicki J, Yamada KD (2017). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* **20**: 1160–1166.

Kochman JMT (1970). *Grzyby (Mycota); Glonowce (Phycomycetes), Wroslikowe (Peronosporales)*. Polska Akademia Nauk, Instytut Botaniki, Poland.

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**: 1870–1874.

Liberato JR, Forsberg L, Grice KR, *et al.* (2006). *Peronospora lamii* on *Lamiaceae* in Australia. *Australasian Plant Pathology* **35**: 367–368.

Mcmillan RT (1993). First report of downy mildew of *Salvia* in Florida. *Plant Disease* **78**: 317.

Meusel HJE (1994). *Vergleichende Chronologie der Zentraleuropäischen Flora, Band II*. Jena, Gustav Fischer Verlag, Germany.

Mulenko W, Majewsky T, Ruszkiewicz-Michalska M (2008). *A preliminary checklist of micromycetes in Poland*. W. Szafer Institute of Botany, Poland.

Müller J, Kokes P (2008). Erweitertes Verzeichnis der Falschen Mehltaupilze Mährens und tschechisch Schlesiens. *Czech Mycology* **60**: 91–104.

Oescu CV, Radulescu IM (1939). Péronosporacées récoltées dans la dépression de la rivière Jijia. III. *Annales des Sciences de la Universitée de Jassy* **24**: 94–104.

Osipjan LL (1967). *Peronosporovye Griby*. Erevanskij Gosudarstvennyj Universitet, Armenia.

Palmateer AJ, Harmon PF, Schubert TS (2008). Downy mildew of coleus (*Solenostemon scutellarioides*) caused by *Peronospora* sp. in Florida. *Plant Pathology* **57**: 372.

Plenk A (2002). *Peronospora lamii* A. Braun, eine noch in Österreich seltene Krankheit an *Salvia officinalis*. In. *Proceedings of the ALVA Meeting*. Vienna, Austria.

Ploch S, Choi Y-J, Rost C, *et al.* (2010). Evolution of diversity in *Albugo* is driven by high host specificity and multiple speciation events on closely related *Brassicaceae*. *Molecular Phylogenetics and Evolution* **57**: 812–820.

Preece TF (2002). *A Checklist of Downy Mildews (Peronosporaceae) of the British Isles*. British Mycological Society, UK.

Rabenhorst GL (1857). *Klotzschii herbarium vivum mycologicum sistens fungorum per totam Germaniam crescentium collectionem perfectam. Editio nova. Centuria VI*. Dresden, Germany.

Rambaut A (1996). *Se-Al: Sequence Alignment Editor, 2.0a11* Available: http://evolve.zoo.ox.ac.uk and http://tree.bio.ed.ac.uk/software/seal/.

Ronquist F, Huelsenbeck JP (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Runge F, Choi Y-J, Thines M (2011). Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. *European Journal of Plant Pathology* **129**: 135–146.

Runge F, Ndambi B, Thines M (2012). Which morphological characteristics are most influenced by the host matrix in downy mildews? A case study in *Pseudoperonospora cubensis*. *PLoS One* **7**: e44863.

Runge F, Thines M (2008). A potential perennial host for *Pseudoperonospora cubensis* in temperate regions. *European Journal of Plant Pathology* **123**: 483–486.

Stamatakis A (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.

Stanjavičenie S (1984). *Peronosporovye griby Pribaltiki.* Vil'njus, Latvia.

Telle S, Thines M (2008). Amplification of McM2 (approximately 620 bp) from 2 mg of up to 129 years old herbarium specimens, comparing 19 extraction methods and 15 polymerases. *PLoS One* **3**: e3584.

Thines M (2019). An evolutionary framework for host shifts - jumping ships for survival. *New Phytologist* **224**: 605–617.

Thines M, Choi Y-J (2016). Evolution, diversity, and taxonomy of the *Peronosporaceae*, with focus on the genus *Peronospora*. *Phytopathology* **106**: 6–18.

Thines M, Telle S, Ploch S, *et al.* (2009). Identity of the downy mildew pathogens of basil, coleus, and sage with implications for quarantine measures. *Mycological Research* **113**: 532–540.

USDA (1960). *Index of plant diseases in the United States*. USDA, USA.

Voglmayr H, Montes-Borrego M, Landa BB (2014). Disentangling *Peronospora* on *Papaver*: phylogenetics, taxonomy, nomenclature and host range of downy mildew of opium poppy (*Papaver somniferum*) and related species. *PLoS One* **9**: e96838.

Walker JB, Sytsma KJ (2007). Staminal evolution in the genus *Salvia* (*Lamiaceae*): molecular phylogenetic evidence for multiple origins of the staminal lever. *Annals of Botany* **100**: 375–391.

Will M, Classen-Bockhoff R (2014). Why Africa matters: evolution of Old World *Salvia* (*Lamiaceae*) in Africa. *Annals of Botany* **114**: 61–83.

Yerkes WD, Shaw CG (1959). Taxonomy of the *Peronospora* species on *Cruciferae* and *Chenopodiaceae*. *Phytopathology* **49**: 499–507.

